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## ORIGINAL ARTICLE

# De novo variants in *KLF7* are a potential novel cause of developmental delay/intellectual disability, neuromuscular and psychiatric symptoms

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Due to small numbers of reported patients with pathogenic variants in single genes, the phenotypic spectrum associated with genes causing neurodevelopmental disorders such as intellectual disability (ID) and autism spectrum disorder is expanding. Among these genes is *KLF7* (Krüppel-like factor 7), which is located at 2q33.3 and has been implicated in several developmental processes. *KLF7* has been proposed to be a candidate gene for the phenotype of autism features seen in patients with a 2q33.3q34 deletion. Herein, we report 4 unrelated individuals with de novo *KLF7* missense variants who share similar clinical features of developmental delay/ID, hypotonia, feeding/swallowing issues, psychiatric features and neuromuscular symptoms, and add to the knowledge about the phenotypic spectrum associated with *KLF7* haploinsufficiency.

## KEYWORDS

autism, clinical diagnostics, intellectual disability, *KLF7*, Krüppel-like transcription factors, whole-exome sequencing, zinc finger DNA-binding protein

## 1 | INTRODUCTION

De novo genetic/genomic variants are increasingly acknowledged as a significant etiology of neurodevelopmental disorders such as intellectual disability (ID) and autism spectrum disorder (ASD).<sup>1–3</sup> For genetically heterogeneous disorders such as developmental delay (DD) and ID, diagnostic-exome sequencing (DES) can identify a genetic etiology in up to 30% of cases when traditional genetic testing is inconclusive.<sup>4</sup> During the past decade, substantial advancements have been made in elucidating the genetic causes of neurodevelopmental disorders. However, over half of individuals with neurodevelopmental disorders still do not have an identified genetic etiology.<sup>5,6</sup>

*KLF7* (Krüppel-like factor 7, OMIM 604865), encodes a transcription factor belonging to the KLF family characterized by the presence of zinc coordinating (Zn) di-cysteine: di-histidine motif (C2H2) and sequence homology to the *Drosophila* segmentation gene product Krüppel.<sup>7</sup> *KLF7* has been implicated in several developmental processes and may be involved in the regulation of postmitotic differentiation of progenitor cells, neuronal morphogenesis, and/or phenotype maintenance.<sup>8</sup> Emerging evidence suggests that *KLF7* haploinsufficiency results in a recognizable neurodevelopmental phenotype. Located at 2q33.3, *KLF7* has been proposed to be one of the possible candidate genes for the phenotype associated to the 2q33.3q34 deletion which has been

reported in patients with ASD.<sup>9–12</sup> Additionally, these patients present with microcephaly, hypotonia, psychomotor retardation and mild dysmorphic features.

Herein, we report 4 unrelated affected individuals with de novo missense variants in *KLF7* detected by DES with of DD/ID, neuromuscular and psychiatric complications.

## 1.1 | Clinical report

Clinical characteristics of the 4 patients are summarized in Table 1.

### 1.1.1 | Patient 1

Patient 1 was born at 38 weeks, 6 pounds 10 oz by c-section due to breech position to Ashkenazi Jewish, non-consanguineous parents. The pregnancy was uncomplicated with possible decreased fetal movements. He had jaundice requiring phototherapy due to difficulty feeding and also had torticollis. The patient was seen by neurology and began occupational and physical therapy at 6 months of age due to diffuse muscle weakness. He rolled over at 6 months of age, sat unassisted at 11 months and began crawling and pulling to stand at 15 months with the aid of therapies. Vision and hearing evaluations at 8 months was normal. At approximately 1 year, he had mildly elevated creatine kinase levels (353 and 419 units per liter). He was evaluated in genetics clinic at 15 months for possible myopathy due to gross motor delay, proximal muscle weakness and positive Gower's sign. At 21 months, he wore ankle braces for support in ambulation, had mild torticollis, pes planus, positive Gower's sign, and mildly wide-based gait. He did not cooperate with formal strength testing at that time. Language was reported to be mildly delayed and there were reports of some difficulty in swallowing. Muscle weakness later resolved; however, he remained hypotonic with language and cognitive delays. Developmental testing at 3 years 7 months showed a language developmental quotient (DQ) of 71% and cognitive DQ of 73%. He was also diagnosed with ASD and anxiety. The patient was non-dysmorphic with normal growth parameters. Brain MRIs at 20 months and 4 years showed stable mild ventriculomegaly. On follow-up evaluation at age 4 years, his muscle tone continued to be low and his running was slow, but he no longer used a Gower's maneuver and strength was normal in upper and lower extremities. Family history was non-contributory. Prior normal genetic testing included *NEB* deletion testing for the p.R2478\_D2512 deletion common in the Ashkenazi Jewish population and *SMN1* copy number testing due to hypotonia.

### 1.1.2 | Patient 2

Patient 2 was initially seen at birth and is currently 16 years old. She was born at 35 weeks at 2 pounds, 12 oz, 36.5 cm, head circumference 29.5 cm (<10th% for gestational age) to Irish, German non-consanguineous parents. Pregnancy and birth history were complicated only by decreased fetal movements. After birth inability to regulate body temperature, hypotonia and feeding difficulties associated with gastroesophageal reflux disease were noted. At 3 weeks of age she developed episodes of apnea that prompted monitoring and recurring hospital admissions. She is reported to be non-verbal, have ID, lower hypertonia, contractures, mild positional scoliosis, cerebral

palsy, strabismus requiring surgery and is described as anxious and shy. Additional features included failure to thrive, short stature, microcephaly, no tear production, little-to-absent sweating and mild dysmorphic features (Figure 1). Family history was non-contributory. Prior normal testing included normal karyotype, chromosomal microarray, congenital disorder of glycosylation testing, methylation for Angelman syndrome and a metabolic work-up.

### 1.1.3 | Patient 3

Patient 3 had been seen by a pediatric neurologist at regular intervals since age 4.5 years. At 15 years old she is reported to have ID (IQ 64), attention deficit disorder/anxiety, neonatal feeding difficulties, motor dyspraxia, upper extremities hypotonia and lower extremity hypertonia. She was reported as "large for her age." Family history was non-contributory. Prior normal testing included normal metabolic screening including a skin biopsy for peroxisomal function, single nucleotide polymorphism (SNP) array, molecular tests for Sotos and Fragile X syndromes, electromyography (EMG) and electroencephalography (EEG).

### 1.1.4 | Patient 4

Patient 4 is a 2 years and 4 months old male with global DD and hypotonia. ID has not been assessed formally, but he spoke his first words at 2 years of age and walked independently at 26 months. He also has hypertelorism. Family history was non-contributory. Prior normal testing included normal metabolic testing and SNP array.

## 2 | METHODS

### 2.1 | Exome sequencing

For all patients, trio whole-exome sequencing was performed with proband and both biological parents on genomic DNA isolated from blood. Diagnostically relevant variants were confirmed by Sanger sequencing. For patient 1, DES was performed by Ambry Genetics Laboratory. Samples were prepared using the SeqCapEZ VCR 2.0 (Roche NimbleGen, Madison, Wisconsin) and sequenced on the Illumina HiSeq 2000 Sequencer (Illumina, San Diego, California). Data annotation and interpretation were performed as previously reported.<sup>4</sup> For patient 2, DES was performed at GeneDx Laboratory with methods as previously reported.<sup>13</sup>

For patients 3 and 4 exome sequencing and variant calling, a parent-offspring trio approach was used as described previously.<sup>3</sup> Briefly, the exome was captured using the Agilent SureSelect v4 kit (Agilent, Santa Clara, California). Exome libraries were sequenced on an Illumina HiSeq2000 instrument (Illumina) with 101 bp paired-end reads at a median coverage of  $\times 75$ . Sequence reads were aligned to the hg19 reference genome using BWA version 0.5.9-r16.<sup>14</sup> Variants were subsequently called by the GATK unified genotyper, version 3.2-2 and annotated using a custom diagnostic annotation pipeline.<sup>15</sup> All variants were classified utilizing ACMG standards and guidelines.<sup>16</sup>

Informed consent was obtained from all patients and family members undergoing sequencing. All research described in this case report was conducted in accordance with the World Medical Association Declaration of Helsinki. The clinical information presented herein

**TABLE 1** Clinical characteristics of patients with *KLF7* variants and deletions

Alteration details	Genotype	Patient 1 (this report)	Patient 2 (this report)	Patient 3 (this report)	Patient 4 (this report)	Courzens et al <sup>9</sup>	Brandau et al <sup>10</sup>	Rosenfeld et al <sup>11</sup>	Jang et al <sup>12</sup>
Alteration details	Genotype	p.T137M c.410C>T	p.D264N c.790G>A	p.P139S c.415C>T	p.T137M c.410C>T	2q33.3q34 deletion, and paternal supernumerary marker der(15)	2q33.3q34 deletion, and de novo apparently balanced (4;16) translocation	2q33.3q34 deletion	2q33.3q34 deletion
Deletion size						NR	1.4–4.4 Mb	6.3 Mb	5.9 Mb
Deletion position						NR	NR	207 584 984–213 908 936	206 048 173–211 980 867
Inheritance		De novo	De novo	De novo	De novo	De novo	De novo	NR	De novo
Gender		Male	Female	Female	Male	Male	Male	NR	Female
Age at last exam		4 y	16 y	15 y	2 y	2 y	6 y	NR	2 y
Micrognathia		None	Mild	None	None	Yes	None	NR	NR
Microcephaly		None	Yes	None	None	Yes	Yes	NR	Yes
Growth retardation		None	None	None, is large for age	None	Yes	None	Yes	None
Birth weight		Normal	Low for 35 wk gestation	NR	NR	Low with IUGR reported	Normal	NR	Low
Motor delay		Yes, although rolled at 6 mo, sat unassisted at 11 mo and began crawling and pulling to stand at 15 mo with therapies	Yes	Yes	Yes, walked at 2 y	NR	Yes, sat at 1 y, first steps at 18–24 mo	Yes	Fine and gross motor delay (<3% ile)
Speech delay		Mildly delayed (71% at 3 y 7/12 y)	Yes, non-verbal at 16 y	Yes, first words at 2 y	Yes, first words at 2 y	NR	Yes, no complete sentences at 6 y	NR	Expressive-receptive language delay (both below first centile)
Cognitive delay		Yes (IQ 73 at 3 y 7/12 y)	Yes	Yes, IQ of 64	Yes, although not formally assessed	Yes, IQ 65 and 58 at 1 y and 2 y 7/12 y	IQ of 51	NR	Yes, moderate intellectual disability
Neuroimaging findings		Mild ventriculomegaly	NR	NR	NR	NR	None	NR	NR
Autism spectrum disorder/Autistic features		None	None	None	None	N/A	Autistic features reported	Autistic features reported	Poor eye contact at 15 mo
Other behavioral/psychiatric features		None	Short attention span and anxiety	Anxiety and ADD	None	NR	Hyperactivity treated with medications and described as “out of control”	NR	Bruxism and repetitive movements
Seizures		None	None	None	None	None	Complex partial seizures	NR	None

(Continues)

TABLE 1 (Continued)

	Patient 1 (this report)	Patient 2 (this report)	Patient 3 (this report)	Patient 4 (this report)	Courtens et al <sup>9</sup>	Brandau et al <sup>10</sup>	Rosenfeld et al <sup>11</sup>	Jang et al <sup>12</sup>
Unsteady gait	Wide-based gait	Abnormal gait	Yes	NR	NR	NR	NR	NR
Hypotonia	Yes, proximal	Yes, centrally	None	Yes	Yes	Yes	NR	Yes
Other neuromuscular symptoms	Previous positive gower sign (resolved before age 4), muscle strength in proximal muscles normal at age 4	Increased lower tone, contractures, mild positional scoliosis, cerebral palsy, decreased fetal movements	Decreased tone in upper extremities, increased tone in lower extremities	None	None	None	NR	None
Craniofacial dysmorphism	None	Sloping forehead, midface hypoplasia	None	None	High forehead, prominent beaked nose, absent earlobes	2 posterior hair whorls, epicanthal folds, high forehead, ptosis, prominent nasal tip and dental anomalies	NR	Down-slanting palpebral fissures, high forehead, small mouth, high palate
Other dysmorphic feature	None	None	None	None	Fifth finger clinodactyly, wide gap between first and second toes	Fifth finger clinodactyly, 2,3 syndactyly, undescended testes, hypospadias	NR	None
Feeding issues	Yes	Yes, continue at age 16 with G-tube and Nissen fundoplication	Yes, as a neonate	None	Yes, with Failure to Thrive reported	Yes	NR	None
Other anomalies	None	Prolonged dysautonomia, asthma, strabismus requiring surgery	None	None	None	None	NR	None

Abbreviation: NR, not reported.

was collected during the routine clinical care of a patient and this study is exempt from Institutional Research Board approval. All subjects participating in this study provided signed, written consent allowing for the publication of their clinical photographs and/or data. In the case of minors, signed written consent was provided by their parents or legal guardians.

Individuals were identified through GeneMatcher.<sup>17</sup>

## 2.2 | Structural analysis

The structure of KLF7 with bound DNA was modeled based on the crystal structure of KLF4 bound to DNA (PDB: 4M9E).<sup>18</sup> The Zinc finger domains of KLF4 and KLF7 share 73% identity, with 100% conservation observed at DNA interacting residues. The program Coot<sup>19</sup> was used to change the amino acids that differed between the sequences of KLF4 and KLF7, most of which were solvent-exposed, and reasonable sidechain rotamers were chosen manually. In silico mutagenesis of Asp 264 to Asn was carried out using FoldX.<sup>20</sup> Model graphics were generated using Pymol (The PyMOL Molecular Graphics System, Version 1.7.1 Schrödinger, LLC.). Motifs were identified using the web server ELM.<sup>21</sup> Disorder was predicted by IUPRED and GlobPlot.<sup>22</sup> Low sequence complexity was predicted by SMART.<sup>23,24</sup>

## 3 | RESULTS

We report patients from 4 unrelated families with a similar phenotype found to have probably gene damaging de novo variants in *KLF7*. The genotypes and analysis of the variants are presented in Figure 1. All sequence variants are described in reference to RefSeq

transcript NM\_003709. In patients 1 and 4, DES independently identified the heterozygous de novo missense variant *KLF7* c.410C>T (p.T137M). In patients 2 and 3, DES identified the heterozygous de novo missense variants *KLF7* c.790G>A (p.D264N) and c.415C>T (p.P139S), respectively.

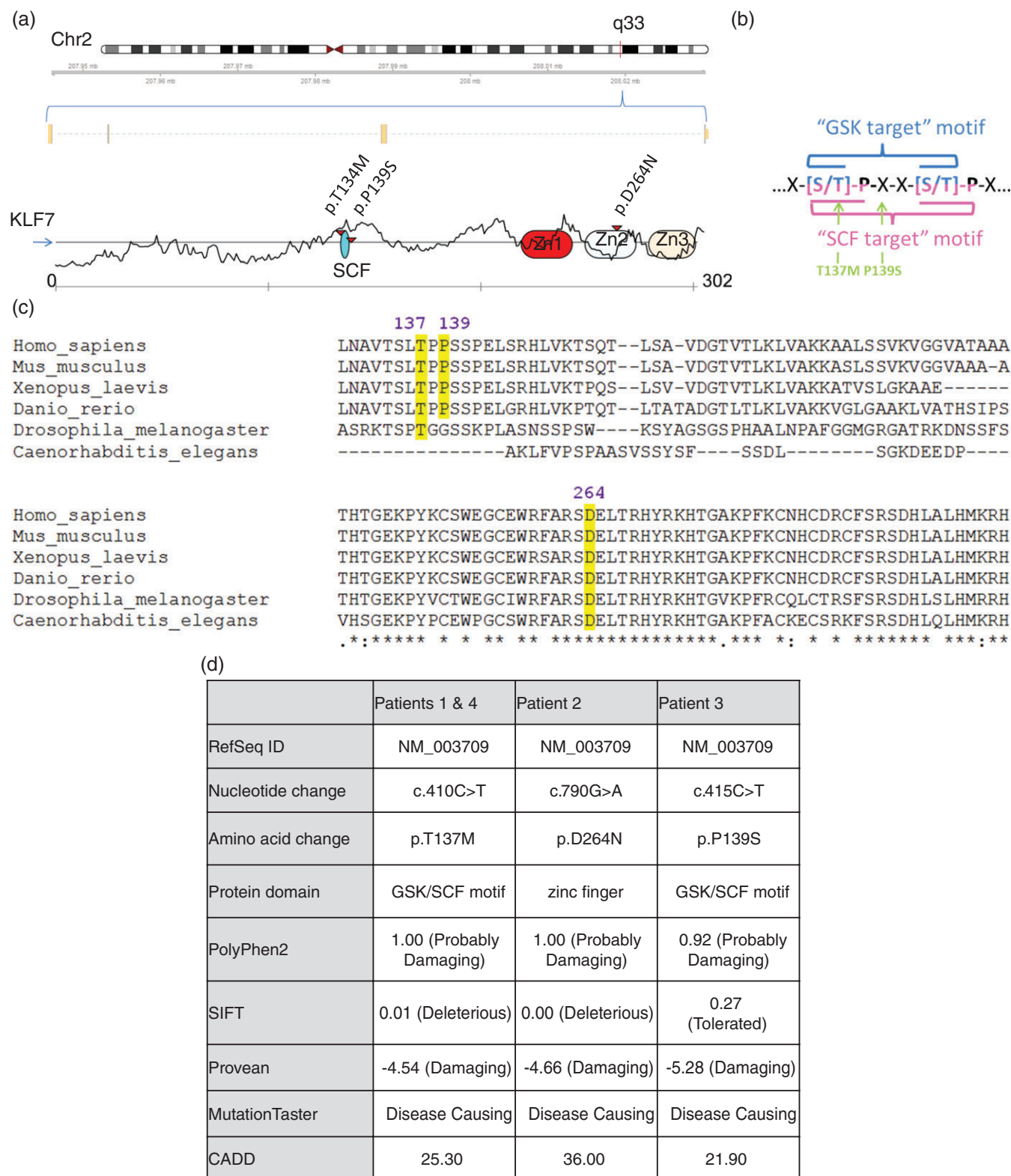
### 3.1 | Structural interpretation of the variants

The *KLF7* c.410C>T (p.T137M) and c.415C>T (p.P139S) variants lie in a flexible, unstructured sequence, in the N-terminal regulatory region of *KLF7*, whereas the c.790G>A (p.D264N) variant is located in the C-terminal zinc finger-containing DNA-binding domain (Figure 2A). The 3 *KLF7* variants are all in highly conserved amino acid positions and are not present in the ExAC database.<sup>25</sup> The 2 N-terminal variants are contained in a span containing 2 noteworthy linear motifs—"Skp1--Cullin--Fbox-WD40 (SCF) ubiquitin ligase target" motif and a "glycogen synthase kinase (GSK) target" motif (Figure 2B). The "GSK target" motif—defined by 2 serine (S) or threonine (T) residues spaced 4 residues apart ([S/T]-X-X-X-[S/T])—identifies the protein to be phosphorylated by GSK. Within this GSK motif is the "SCF ubiquitin ligase target" motif, defined as the GSK motif where the positions immediately after each S/T are proline ([S/T]-P-X-X-[S/T]-P). Upon phosphorylation at both S/T positions by the kinase, this site would be recognized by the SCF ubiquitination complex, which promotes degradation. In the paralog *KLF2*, which has the same motif, direct variant of these motifs negatively impacted its SCF-mediated degradation in vitro and in vivo indicating a key biological role for this sequence.<sup>26</sup> In *KLF7*, the variant c.410C>T (p.T137M) sits within the motifs and is anticipated to disrupt this motif by eliminating a phosphorylation site; on the other hand there is no evidence that,



**FIGURE 1** Patient 2 at (A) 3 years, (B) 8 years, (C) 10 years, (D) 12 years, and (E) 17 years





**FIGURE 2** (A) *KLF7* Gene Structure-Ideogram of the *KLF7* centered by the gene structure and the protein architecture on the bottom. The arrow line demarks the prediction (shown as a squiggle line) of the proteins tendency to be disordered (squiggle above the line) or structured (squiggle below the line). The zinc-finger (C2H2 type) domains are label Zn1, Zn2, and Zn3, respectively. The Skp1--Cullin--Fbox-WD40 (SCF) is also indicated. Observed variants are indicated by red triangles. (B) *KLF7* Structural Motif. GSK, glycogen synthase kinase. (C) conservation of variants, and (D) in silico predictions

c.415C>T (p.P139S) would impact the motif and probably mechanism of disruption of this variant cannot be elucidated from available information, suggesting that this region warrants deeper functional investigation. The third variant, c.790G>A (p.D264N), is located in the

C-terminal zinc finger-containing DNA-binding domain, shown to bind to the minimal enhancer of the *TrkA* gene, which encodes the high affinity receptor for nerve growth factor. This variant involves one of the specificity residues of the zinc-finger domain involved in



**FIGURE 3** The interactions of KLF7 with target DNA sequence. (A) Schematic representation of interactions between the Zinc fingers of KLF7 and DNA, with region of interest emphasized. Each position shows possible recognized bases as determined experimentally for KLF4. Bases present in crystal structure bolded. Bases defined specifically by interactions shown in red. Hydrogen-bonding and electrostatic interaction in region of interest shown with red arrows. (B) Structural representation of interactions involving D264, and predicted interactions involving N264. Hydrogen bonds and electrostatic interactions shown with dashed yellow lines. D264 is one of the specificity residues of the Zinc-finger involved in interactions with DNA. An variant of this residue is probably to shift DNA-binding selectivity due to altered interaction possibilities, which may result in impaired activation of target genes

interactions with DNA. An variant of this residue is probably to shift DNA binding selectivity due to altered interaction possibilities, which may result in impaired activation of target genes which has been observed in the zinc-finger proteins (Figure 3).

## 4 | DISCUSSION

We report patients from 4 unrelated families with a similar phenotype found to have probably gene damaging de novo variants in *KLF7*. Among the 3 different de novo missense variants identified in our cohort, there is one heterozygote with the p.T137M in GnomAD, and one heterozygote with p.P139L (a different substitution at same residue) in GnomAD.<sup>25</sup> An additional female patient with ID was reported through ClinVar, carrying the same de novo variant as our patient 2 (ClinVar submission accession number SCV000493088.1). The variant was reported as of uncertain clinical significance; no other details were available. While the individuals reported in GnomAD are not expected to have early-onset severe disorder, due to the variability present in patients 1 and 4, it is possible that this individual might be mildly affected. Without further analysis of this variant in additional individuals, a potential a female protective model cannot be ruled out.<sup>27</sup>

#### 4.1 | Phenotype of patients with KLF7 variants and 2q33.3q34deletion

DD/ID, hypotonia, feeding/swallowing issues, psychiatric features and neuromuscular symptoms were present in 3 patients in our

cohort, with the additional patient showing mild neuromuscular symptoms, DD, but being too young for formal psychiatric testing. The additional individual present in ClinVar was reported with at least ID. These characteristics are similar to the individuals reported with the 2q33.3q34deletion syndrome, although the phenotype is also highly variable (reviewed in Table 1).<sup>9-12</sup> Of the 4 previously reported patients in the literatures with the deletion, varying degrees of DD/ID, hyptonia, feeding/swallowing issues, psychiatric features and neuromuscular issues were reported along with dysmorphic features, seizures and microcephaly. None of the cases in our cohort had seizures. Dysmorphic features were reported in 3 individuals with the overlapping deletion and in one affected individual of our cohort. Growth retardation and microcephaly were reported in 2 individuals with the 2q deletion and one individual in our cohort. In contrast, one individual in our cohort was reported with tall stature and macrocephaly. Ronzoni et al<sup>28</sup> also reported an additional patient with a *KLF7* deletion. This deletion also included more than 50 RefSeq genes; 17 listed as human disease genes in OMIM. Therefore, it is difficult to establish correlation to individuals with *KLF7* only variants especially due to the cumulative nature of these genes, but suggests that *KLF7* does have a role in neurodevelopmental features.

The variability between the 2 patients within our cohort with the same variant may either be explained by the lack of phenotype/genotype connection within *KLF7* or the young age of the patient. Further chronological study of the patients within this cohort and the discovery of additional patients may be helpful in elucidating this construct.



## 4.2 | Pathogenic mechanisms of KLF7 variants

Homozygous *Klf7* null (*Klf7*<sup>-/-</sup>) mice were born at expected Mendelian frequencies but a majority of them (98.5%) died within the first 3 days of life with very little or no milk in their stomachs, hypopnea and cyanosis.<sup>29</sup> Additionally, these mice showed defects in neurite outgrowth, axonal misprojection at specific locations within the nervous system including the olfactory and visual systems, cerebral cortex and hippocampus and reduced dendritic branching in the hippocampus.<sup>29</sup> Abnormal corpus callosum (CC) in the null mouse was seen due to failure of the fibers of CC to cross the midline, a missing or severely disrupted anterior commissure and a reduction in the size of the fimbria.<sup>29,30</sup> Lei et al<sup>30</sup> reported that sensory nociceptive neurons in *Klf7*<sup>-/-</sup> newborn mice are significantly reduced due to increased apoptosis. The authors find that TrkA expression is reduced in the DRG neurons of *Klf7*<sup>-/-</sup> mice because *Klf7* has been shown to bind to a minimal enhancer in the TrkA upstream promoter region.

Using an RNAi-mediated knockdown approach in the neuroepithelial cell line PC12, Caiazzo et al<sup>31</sup> observed that downregulation of KLF7 gene expression caused silencing of genes crucial for neuronal differentiation, namely MAP2 and the high affinity receptor for NGF, TrkA. Downregulation of TrkA caused a failure of shKlf7-PC12 stable clones to differentiate into neurons. KLF7 role in cellular differentiation was not limited to the nervous system. *Klf7*-silenced embryonic stem cells fail to differentiate into cardiomyocytes, and *Klf7*<sup>-/-</sup> mouse embryonic fibroblasts display impaired adipogenesis and enhanced osteogenesis ability.

The roles of proteins in the KLF family have been reviewed extensively and although a few of them have nervous system specific roles, none of them have been implicated in human disease to date.<sup>32</sup> However, mutations affecting KLF7-interacting proteins have been shown to cause genetic diseases in humans. For example, a dominant mutation in the *FBXO38* gene, encoding the F-box protein 38, has been reported in members of 2 unrelated families affected by a distinct form of distal spinal muscular atrophy which initially manifests as calf weakness.<sup>33</sup> *FBXO38* is a transcriptional coactivator of KLF7 and the authors report that this mutation led to dysregulation of KLF7 target genes and impairment of neurite outgrowth in primary motor neurons indicating a critical role for *FBXO38* and KLF7 in axonal development and neuronal maintenance. One of the KLF7 target genes is *L1CAM*, encoding the neural cell adhesion molecule L1 that plays essential roles in neuronal migration, axon growth and guidance and synaptic plasticity in both the central and peripheral nervous systems.<sup>34</sup> X-linked recessive mutations in the *L1CAM* gene have been shown to cause hydrocephalus, a phenotype associated with enlarged cerebral ventricles, mental retardation and often with spastic paraparesis and adducted thumbs (OMIM: 307000).

## 5 | CONCLUSION

In summary, we present the clinical phenotype information of 4 patients with de novo *KLF7* variants, supporting the relationship of the gene with neurodevelopmental features. We hypothesize that the core phenotype of DD/ID, neuromuscular and psychiatric symptoms is present in individuals with *KLF7* variants along with potential

additional features. We report the varying phenotype of the individuals with these variants. It is anticipated that additional individuals with *KLF7* variants will be identified, adding to the knowledge of the clinical spectrum and pathogenicity. Additional studies on patients with *KLF7* variants and functional studies of these variants are necessary to further elucidate the correlation between genotype and phenotype, preferably supported by further investigations of the molecular mechanism of KLF7 during neurological development and function.

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## Conflict of interest

Zöe Powis, Igor Petrik, Robert Huether, Sha Tang and Deepali N. Shinde are employed and receive a salary from Ambry Genetics. Rashmi Chikarmane and Amber Begtrup are employed and receive a salary from GeneDx. Julie S. Cohen is a consultant to Invitae. Exome sequencing is a commercially available test.

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